

D E C L A R A T I O N

I, SHINICHI USUI, a Japanese Patent Attorney registered No. 9694, of Okabe International Patent Office at No. 602, Fuji Bldg., 2-3, Marunouchi 3-chome, Chiyoda-ku, Tokyo, Japan, hereby declare that I have a thorough knowledge of Japanese and English languages, and that the attached pages contain a correct translation into English of only the claim part of the priority documents of Japanese Patent Application No. 2002-309786 filed on October 24, 2002 in the name of CANON KABUSHIKI KAISHA.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 14th day of February, 2008



SHINICHI USUI

PATENT OFFICE
JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy
of the following application as filed with this office.

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Commissioner,
Patent Office Yasuo IMAI

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Applicant's Information

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[Addressed to] Commissioner of the Patent Office

[International Classification] C08G 63/02

[Title of the Invention] NEW POLYHYDROXYALKANOATE COMPRISING UNIT HAVING (PHENYLEMTHYL)OXY STRUCTURE ON SIDE CHAIN, AND METHOD FOR PRODUCING THE SAME

[Number of the Claims] 20

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[Indication of Official Fee]

[Prepayment Ledger No.] 011224

[Amount] ¥21000

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[Material] Drawings 1

[Material] Abstract 1

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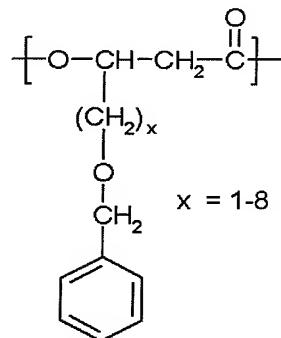
NEW POLYHYDROXYALKANOATE COMPRISING UNIT HAVING
(PHENYLMETHYL) OXY STRUCTURE ON SIDE CHAIN, AND METHOD
5 FOR PRODUCING THE SAME

[Claims]

[Claim 1]

A polyhydroxyalkanoate comprising a 3-hydroxy-
10 ω -[(phenylmethyl)oxy]alkanoic acid unit represented
by a chemical formula (1):

[chemical 1]



(1)

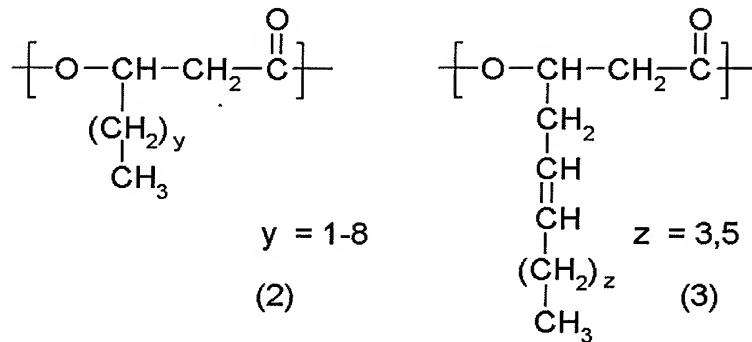
15 (wherein x may assume one or more arbitrary integral values within the range shown in the chemical formula).

[Claim 2]

20 The polyhydroxyalkanoate according to claim 1,
comprising, in addition to the unit represented by
the chemical formula (1), at least one of the units

represented by chemical formulae (2) and (3):

[chemical 2]

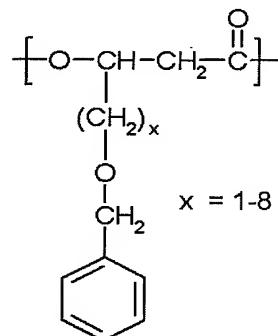


(wherein y and z each may assume one or more arbitrary integral values within the range shown in the chemical formula, independently from the unit represented by the chemical formula (1)).

[Claim 3]

10 The polyhydroxyalkanoate according to claim 1 or 2, comprising, simultaneously in a molecule thereof, at least the 3-hydroxy- ω -[(phenylmethyl)oxy]alkanoic acid unit represented by the chemical formula (1):

15 [chemical 3]

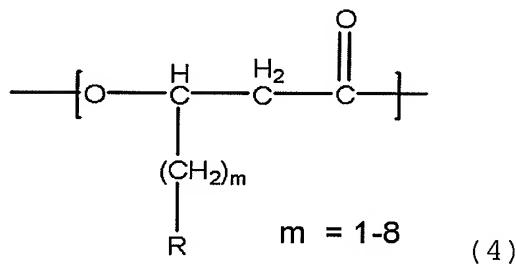


(1)

(wherein x may assume one or more arbitrary integral values within the range shown in the chemical formula);

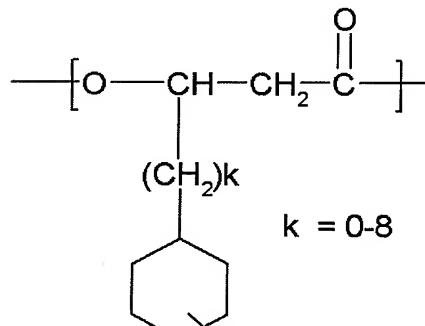
5 and a 3-hydroxy- ω -cyclohexylalkanoic acid unit represented by a chemical formula (4):

[chemical 4]



(wherein m may assume one or more arbitrary integral values within the range shown in the chemical formula; and R includes a residue having either a phenyl structure or thienyl structure),
10 or represented by a chemical formula (5):

[chemical 5]



15 (wherein R1 represents a substituent on a cyclohexyl

group and R1 is a H atom, a CN group, a NO2 group, a

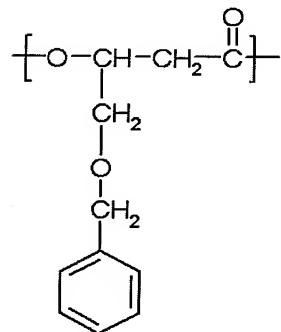
halogen atom, a CH₃ group, a C₂H₅ group, a C₃H₇ group, a CF₃ group, a C₂F₅ group or a C₃F₇ group, and k may assume one or more arbitrary integral values within the range shown in the chemical formula).

5

[Claim 4]

The polyhydroxyalkanoate according to any one of claims 1 to 3, wherein the 3-hydroxy- ω -[(phenylmethyl)oxy]alkanoic acid unit represented by 10 the chemical formula (1) is either one or more of: a 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid unit represented by a chemical formula (6):

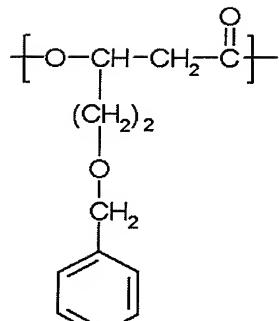
[chemical 6]



(6)

15 and a 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid unit represented by a chemical formula (7):

[chemical 7]

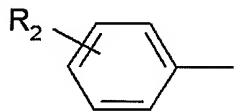


(7)

[Claim 5]

The polyhydroxyalkanoate according to claim 3 or 4, wherein R in the chemical formula (4), namely 5 the residue having a phenyl structure or a thiienyl structure belongs to a group of residues represented by a chemical formula (8):

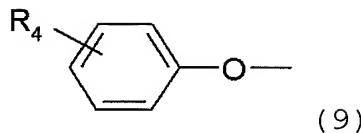
[chemical 8]



(8)

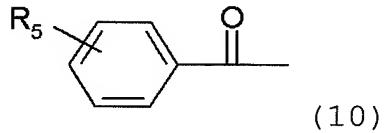
10 (wherein R₂ indicates a substituent group on the aromatic ring and R₂ represents a H atom, a halogen atom, a CN group, a NO₂ group, a CH₃ group, a C₂H₅ group, a C₃H₇ group, a CH=CH₂ group, a COOR₃ group (wherein R₃ represents any one of a H atom, a Na atom 15 and a K atom), a CF₃ group, a C₂F₅ group or a C₃F₇ group, and in a case where plural units are present, R₂ may be different for each unit); a group of residues represented by a chemical formula (9):

[chemical 9]



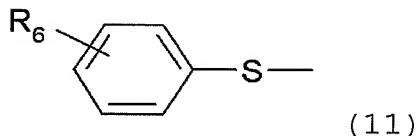
(wherein R_4 indicates a substituent group on the aromatic ring and R_4 represents a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a SCH_3 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and in a case where plural units are present, R_4 may be different for each unit; a group of residues represented by a chemical formula 10):

[chemical 10]



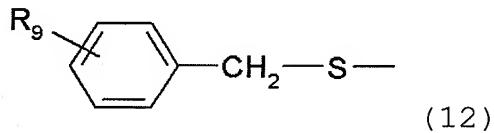
(wherein R_5 indicates a substituent group on the aromatic ring and R_5 is a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and in a case where plural units are present, R_5 may be different for each unit); a group of residues represented by a chemical formula (11):

20 [chemical 11]



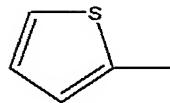
(wherein R_6 indicates a substituent group on the aromatic ring and R_6 represents a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_7$ group, a SO_2R_8 group (wherein R_7 represents any one of H, Na, K, CH_3 and C_2H_5 , and R_8 represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2-CH$ group, or a $(CH_3)_3-C$ group, and in a case where plural units are present, R_6 may be different for each unit); a group of residues represented by a chemical formula (12):

[chemical 12]



(wherein R_9 represents a substituent group on the aromatic ring, and R_9 represents a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{10}$ group, a SO_2R_{11} group (wherein R_{10} represents any one of H, Na, K, CH_3 and C_2H_5 , and R_{11} represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2-CH$ group or a $(CH_3)_3-C$ group, and in a case where plural units are present, R_9 may be different for each unit); a group of residues represented by a chemical formula (13):

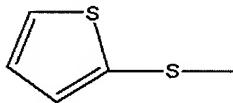
[chemical 13]



(13)

a group of residues represented by a chemical formula (14):

[chemical 14]

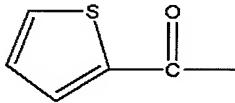


(14);

5

a group of residues represented by a chemical formula (15):

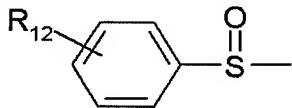
[chemical 15]



(15)

10 a group of residues represented by a chemical formula (16):

[chemical 16]

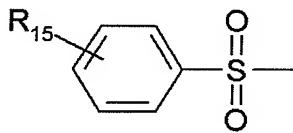


(16)

15 (wherein R₁₂ indicates a substituent group on the aromatic ring and R₁₂ represents any one of a H atom, a halogen atom, a CN group, a NO₂ group, a COOR₁₃ group, a SO₂R₁₄ group (wherein R₁₃ represents any one of H, Na, K, CH₃ and C₂H₅, and R₁₄ represents any one of OH, ONa, OK, a halogen atom, OCH₃ and OC₂H₅), a CH₃

group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2-CH$ group and $(CH_3)_3-C$ group, and in a case where plural units are present, R_{12} may be different for each unit); a group of residues represented by a chemical formula 5 (17):

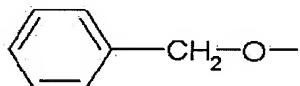
[chemical 17]



(17)

(wherein R_{15} indicates a substituent group on the aromatic ring and R_{15} is any one of a H atom, a 10 halogen atom, a CN group, a NO_2 group, a $COOR_{16}$ group, a SO_2R_{17} group (wherein R_{16} represents any one of H, Na, K, CH_3 and C_2H_5 , and R_{17} represents any one of OH, ONa , OK , a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2-CH$ group and a 15 $(CH_3)_3-C$ group, and in a case where plural units are present, R_{15} may be different for each unit); and a group of residues represented by a chemical formula (18):

[chemical 18]



20 (18)

[Claim 6]

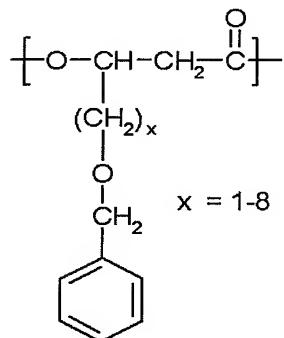
The polyhydroxyalkanoate according to any one

of claims 1 to 5, wherein a number-average molecular weight is within a range from 1,000 to 1,000,000.

[Claim 7]

5 A method for producing a polyhydroxyalkanoate containing, in a molecule thereof, a 3-hydroxy- ω -[(phenylmethyl)oxy]alkanoic acid unit represented by a chemical formula (1):

[chemical 21]

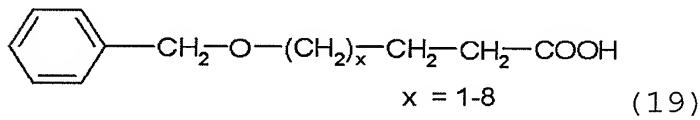


10

(wherein x may assume one or more arbitrary integral values within the range shown in the chemical formula), which comprises allowing, under a condition containing ω -[(phenylmethyl)oxy]alkanoic acid represented by a chemical formula (19):

15

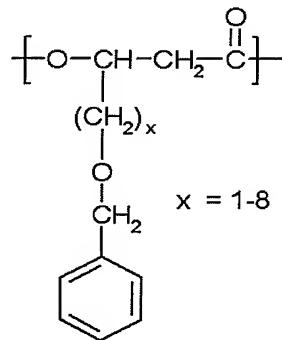
[chemical 19]



(wherein x may assume one or more arbitrary integral values within the range shown in the chemical

formula), a microorganism having an ability to produce a polyhydroxyalkanoate containing in a molecule thereof a 3-hydroxy- ω -[(phenylmethyl)oxy]alkanoic acid unit of the chemical 5 formula (1):

[chemical 20]

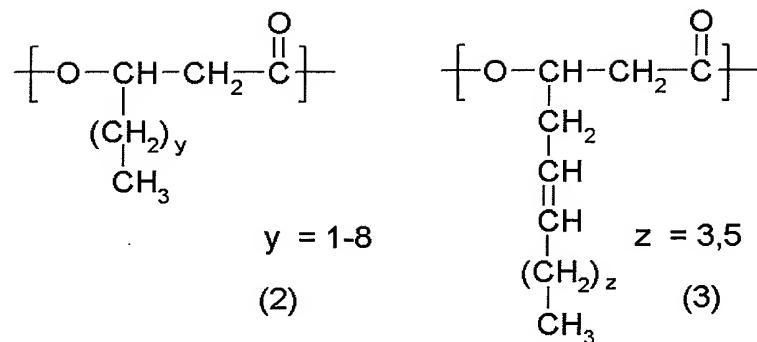


to execute biosynthesis.

10 [Claim 8]

The method for producing a polyhydroxyalkanoate according to claim 7, wherein polyhydroxyalkanoate contains, in addition to the unit represented by the chemical formula (1), at least one of the units 15 represented by chemical formulae (2) and (3):

[chemical 22]

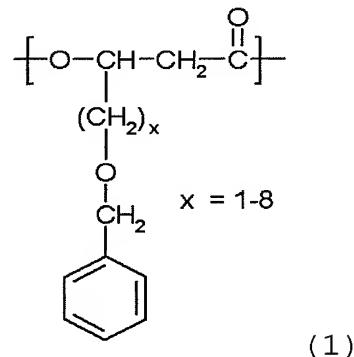


(wherein y and z each may assume one or more arbitrary integral values within the range shown in the chemical formulae, independently from the unit 5 represented by the chemical formula (1)).

[Claim 9]

The producing method according to claim 7 or 8 for producing a polyhydroxyalkanoate containing, 10 within a molecule at the same time, a 3-hydroxy- ω -[(phenylmethyl)oxy] alkanoic acid unit represented by a chemical formula (1):

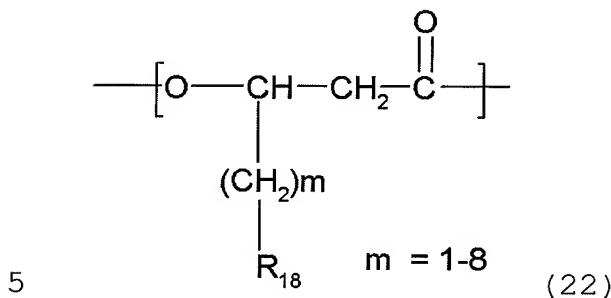
[chemical 29]



15 (wherein x may assume one or more arbitrary integral

values within the range shown in the chemical formula), and a 3-hydroxy- ω -cyclohexylalkanoic acid unit represented by a chemical formula (22):

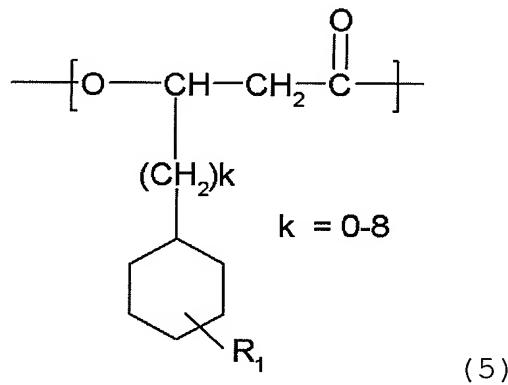
[chemical 30]



(wherein m may assume one or more arbitrary integral values within the range shown in the chemical formula; and R_{18} includes a residue having either a phenyl structure or thienyl structure),

10 or represented by a chemical formula (5):

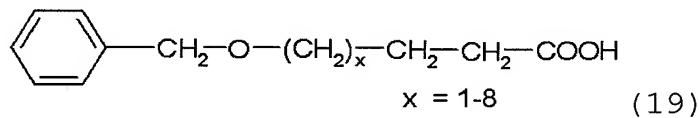
[chemical 31]



15 (wherein R_1 represents a substituent on a cyclohexyl group and R_1 is a H atom, a CN group, a NO_2 group, a halogen atom, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and k may

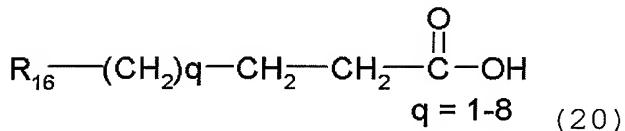
assume one or more arbitrary integral values within the range shown in the chemical formula), the method comprising, under a condition containing ω -[(phenylmethyl)oxy]alkanoic acid represented by a
5 chemical formula (19):

[chemical 23]



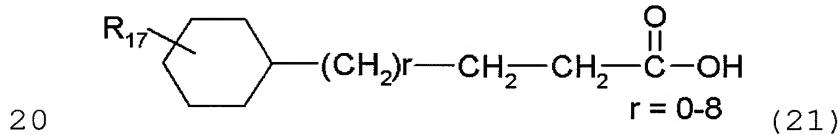
(wherein x may assume one or more arbitrary integral values within the range shown in the chemical
10 formula) and ω -cyclohexylalkanoic acid represented by a chemical formula (20)

[chemical 24]



(wherein q may assume one or more arbitrary integral
15 values within the range shown in the chemical formula; and R_{16} includes a residue having either a phenyl structure or thienyl structure), or represented by:

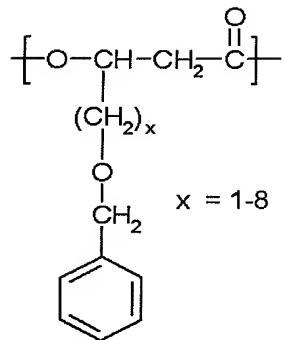
[chemical 25]



(wherein R_{17} represents a substituent on a cyclohexyl

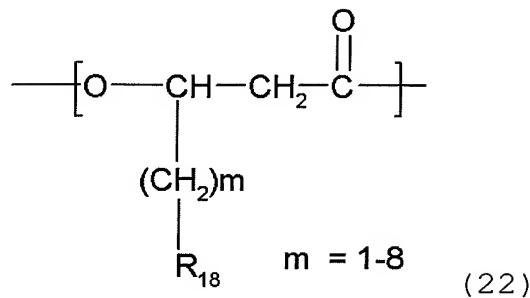
group and R_{17} is a H atom, a CN group, a NO_2 group, a halogen atom, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and r may assume one or more arbitrary integral values within
5 the range shown in the chemical formula), utilizing
 ω -[(phenylmethyl)oxy] alkanoic acid represented by
the chemical formula (19) and the compound
represented by the chemical formula (20) or ω -
10 cyclohexylalkanoic acid represented by the chemical
formula (21) as the raw material and executing a
biosynthesis by a microorganism having an ability to
produce a polyhydroxyalkanoate including, in a
molecule thereof at the same time, a 3-hydroxy- ω -
15 [(phenylmethyl)oxy] alkanoic acid unit represented by
the chemical formula (1):

[chemical 26]



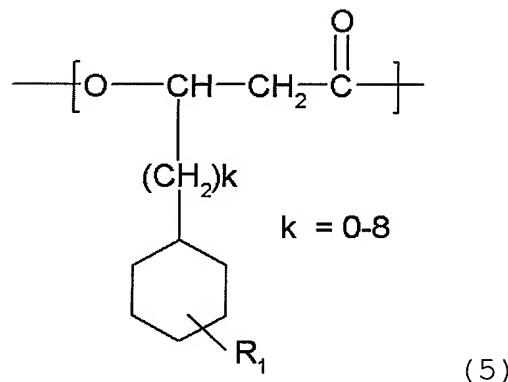
(wherein x may assume one or more arbitrary integral
values within the range shown in the chemical
20 formula) and a 3-hydroxy- ω -cyclohexylalkanoic acid
unit represented by a chemical formula (22)

[chemical 27]



(wherein m may assume one or more arbitrary integral values within the range shown in the chemical formula; and R_{18} includes a residue having either a phenyl structure or thiaryl structure), or represented by a chemical formula (5):

[chemical 28]

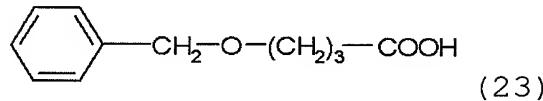


10 (wherein R_1 represents a substituent on a cyclohexyl group and R_1 is a H atom, a CN group, a NO_2 group, a halogen atom, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and k may assume one or more arbitrary integral values within 15 the range shown in the chemical formula).

[Claim 10]

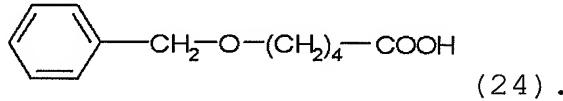
The method for producing a polyhydroxyalkanoate according to any one of claims 7 to 9, wherein the ω -[(phenylmethyl)oxy]alkanoic acid represented by said 5 chemical formula (19) is 4-[(phenylmethyl)oxy]butyric acid represented by a chemical formula (23):

[chemical 32]



10 or 5-[(phenylmethyl)oxy]valeric acid represented by a chemical formula (24):

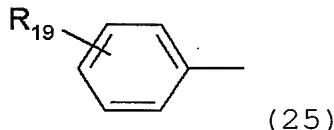
[chemical 33]



[Claim 11]

15 The method for producing a polyhydroxyalkanoate according to claim 9 or 10, wherein R_{16} in the chemical formula (20) and R_{18} in the chemical formula (22), namely the residues having a phenyl structure or a thienyl structure, belong to a group of residues 20 represented by a chemical formula (25):

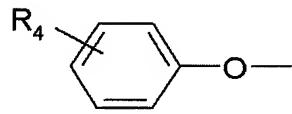
[chemical 34]



(wherein R_{19} indicates a substituent group on the

aromatic ring and R_{19} represents a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $CH=CH_2$ group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and in a case where plural 5 units are present, R_{19} may be different for each unit); a group of residues represented by a chemical formula (9):

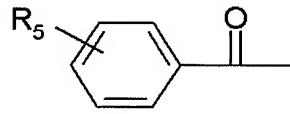
[chemical 35]



(9)

10 (wherein R_4 indicates a substituent group on the aromatic ring and R_4 represents a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a SCH_3 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and in a case where plural 15 units are present, R_4 may be different for each unit); a group of residues represented by a chemical formula (10):

[chemical 36]

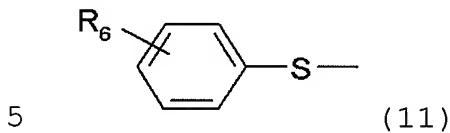


(10)

20 (wherein R_5 indicates a substituent group on the aromatic ring and R_5 is a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group,

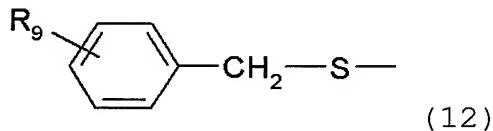
and in a case where plural units are present, R_6 may be different for each unit); a group of residues represented by a chemical formula (11):

[chemical 37]



(wherein R_6 indicates a substituent group on the aromatic ring and R_6 represents a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_7$ group, a SO_2R_8 group (wherein R_7 represents any one of H, Na, K, CH_3 and C_2H_5 , and R_8 represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2-CH$ group, or a $(CH_3)_3-C$ group, and in a case where plural units are present, R_6 may be different for each unit); a group of residues represented by a chemical formula (12):

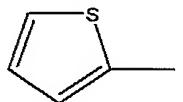
[chemical 38]



(wherein R_9 represents a substituent group on the aromatic ring, and R_9 represents a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{10}$ group, a SO_2R_{11} group (wherein R_{10} represents any one of H, Na, K, CH_3 and C_2H_5 , and R_{11} represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5

group, a C_3H_7 group, a $(CH_3)_2-CH$ group or a $(CH_3)_3-C$ group, and in a case where plural units are present, R_9 may be different for each unit); a group of residues represented by a chemical formula (13):

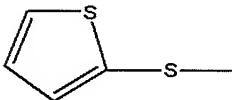
5 [chemical 39]



(13)

a group of residues represented by a chemical formula (14):

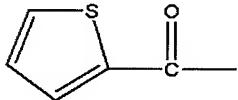
[chemical 40]



10 (14);

a group of residues represented by a chemical formula (15):

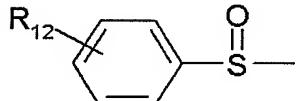
[chemical 41]



(15)

15 a group of residues represented by a chemical formula (16):

[chemical 42]



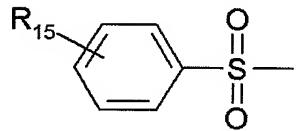
(16)

(wherein R_{12} indicates a substituent group on the

aromatic ring and R_{12} represents any one of a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{13}$ group, a SO_2R_{14} group (wherein R_{13} represents any one of H, Na, K, CH_3 and C_2H_5 , and R_{14} represents any one of OH, ONa , OK , a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2-CH$ group and $(CH_3)_3-C$ group, and in a case where plural units are present, R_{12} may be different for each unit); a group of residues represented by a chemical formula

5 (17) :

[chemical 43]

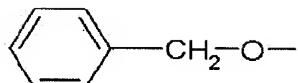


(17)

(wherein R_{15} indicates a substituent group on the aromatic ring and R_{15} is any one of a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{16}$ group, a SO_2R_{17} group (wherein R_{16} represents any one of H, Na, K, CH_3 and C_2H_5 , and R_{17} represents any one of OH, ONa , OK , a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2-CH$ group and a $(CH_3)_3-C$ group, and in a case where plural units are present, R_{15} may be different for each unit); and a group of residues represented by a chemical formula

10 (18) :

[chemical 44]



(18)

[Claim 12]

The method for producing a polyhydroxyalkanoate
5 according to any one of claims 7 to 11, wherein the
microorganism is cultured in a medium containing the
ω-[(phenylmethyl)oxy]alkanoic acid represented by
chemical formula (19).

10 [Claim 13]

The method for producing a polyhydroxyalkanoate
according to any one of claims 9 to 11, wherein the
microorganism is cultured in a medium containing the
ω-[(phenylmethyl)oxy]alkanoic acid represented by
15 chemical formula (19) and the compound represented by
the chemical formula (20) or the ω-cyclohexylalkanoic
acid represented by chemical formula (21).

[Claim 14]

20 The method for producing a polyhydroxyalkanoate
according to claim 12 or 13, wherein the
microorganism is cultured in a medium containing, in
addition to ω-[(phenylmethyl)oxy]alkanoic acid
represented by chemical formula (19), at least one of
25 peptides, yeast extracts, organic acids or salts

thereof, amino acids or salts thereof, saccharides and straight-chain alkanoic acids containing 4 to 12 carbon atoms or salts thereof.

5 [Claim 15]

The method for producing a polyhydroxyalkanoate according to claim 14, wherein the peptide contained in the culture medium is polypeptone; the organic acids contained in the culture medium or salts thereof are one or more compounds selected from the group consisting of pyruvic acid, oxaloacetic acid, citric acid, isocitric acid, ketoglutaric acid, succinic acid, fumaric acid, malic acid, lactic acid, and salts thereof; the amino acids or salts thereof are one or more compounds selected from the group consisting of glutamic acid, aspartic acid, and salts thereof; and the saccharides contained in the culture medium are one or more compounds selected from the group consisting of glyceroaldehyde, erythrose, 20 arabinose, xylose, glucose, galactose, mannose, fructose, glycerol, erythritol, xylitol, gluconic acid, glucronic acid and galacturonic acid, maltose, sucrose and lactose.

25 [Claim 16]

The method for producing a polyhydroxyalkanoate according to any one of claims 12 to 15, wherein said

culture of microorganisms comprises two or more culturing steps.

[Claim 17]

5 The method for producing a polyhydroxyalkanoate according to claim 16, wherein said culture is a fed-batch culture.

[Claim 18]

10 The method for producing a polyhydroxyalkanoate according to any one of claims 12 to 17, comprising a step of culturing the microorganism in a medium containing ω -[(phenylmethyl)oxy]alkanoic acid represented by chemical formula (19) and recovering
15 polyhydroxyalkanoate containing 3-hydroxy- ω -[(phenylmethyl)oxy]alkanoic acid unit represented by the chemical formula (1) generated by the microorganism from the cells of the microorganism.

20 [Claim 19]

 The method for producing a polyhydroxyalkanoate according to any one of claims 7 to 18, wherein the microorganism belongs to *Pseudomonas* species.

25 [Claim 20]

 The method for producing a polyhydroxyalkanoate according to claim 19, wherein the microorganism is

one or more strains selected from the group consisting of *Pseudomonas cichorii* YN2 (FERM BP-7375), *Pseudomonas cichorii* H45 (FERM BP-7374) and *Pseudomonas jessenii* P161 (FERM BP-7376).